CLAIMS

1. A cDNA-RNA hybrid comprising a first strand cDNA synthesis substantially hybridised to RNA wherein the cDNA comprises an amplifier sequence and an RNA annealing region operably linked to an RNA polymerase promoter, and wherein at least one non-templated nucleotide at the 3' end of the first strand cDNA is hybridised to a template switching oligonucleotide.

2. A cDNA-RNA hybrid according to claim 1 wherein the RNA is mRNA.

3. A cDNA-RNA hybrid according to claim 1 or claim 2 wherein the RNA polymerase promoter is a bacteriophage promoter.

4. A cDNA-RNA hybrid according to claim 3 wherein the bacteriophage promoter is selected from the group consisting of T7, T3 and SP6.

5. A cDNA-RNA hybrid according to claim any one of the preceding claims wherein the RNA annealing region comprises poly (dT).

6. A cDNA-RNA hybrid according to claim 5 wherein the oligo(T) region is from about 10 to about 30 T residues in length.

7. A cDNA-RNA hybrid according to any one of the preceding claims wherein the 3' end of the RNA annealing region comprises a VN clamp, wherein V is A, G or C and N is A, G, C or T.

8. A cDNA-mRNA hybrid according to any one of the preceding claims wherein at least one non-templated nucleotide at the 3' end of the first strand cDNA synthesis is deoxycytidine.

9. A cDNA-mRNA hybrid according to any one of the preceding claims wherein at least three non-templated nucleotide at the 3' end of the first strand cDNA synthesis are hybridised to a template switching oligonucleotide.

10. A cDNA-mRNA hybrid according to any one of the preceding claims wherein at least three of the non-templated nucleotides at the 3' end of the first strand cDNA synthesis are deoxycytidine nucleotides.

11. A cDNA-mRNA hybrid according to any one of the preceding claims wherein the template switching oligonucleotide has at least three guanine residues at its 3' end.

12. A cDNA-mRNA hybrid according to any one of the preceding claims wherein the amplifier sequence, the amplification primer and the template switching oligonucleotide contain the same sequence.

13. A cDNA-mRNA hybrid according to any one of the preceding claims wherein the 3' end of the first strand cDNA synthesis is extended such that it is substantially complementary to the template switching oligonucleotide.

14. A cDNA-mRNA hybrid according to any one of the preceding claims wherein the first strand cDNA synthesis is synthesised by a reverse transcriptase.

15. A cDNA-mRNA hybrid according to claim 14 wherein the reverse transcriptase lacks RNaseH activity but retains wild-type polymerase activity.

16. A cDNA-mRNA hybrid according to claim 14 or claim 15 wherein the reverse transcriptase is a Moloney Murine Leukemia virus (MMLV) reverse transcriptase or a mutant thereof.

17. A cDNA-mRNA hybrid according to any one of claims 14 to 16 wherein the reverse transcriptase is PowerScript™ Reverse Transcriptase (BD Biosciences Clontech).

18. A method for amplifying RNA in a sample comprising the steps of:

(a) providing a cDNA synthesis oligonucleotide comprising an amplifier sequence and an RNA annealing region operably linked to an RNA polymerase promoter;

(b) annealing the RNA annealing region of the cDNA synthesis oligonucleotide to RNA under suitable conditions to produce a cDNA-RNA complex;

(c) incubating said cDNA-RNA complex under conditions which permit template-dependent extension of the cDNA synthesis oligonucleotide to generate an cDNA-RNA hybrid;

(d) contacting said cDNA-mRNA hybrid with a template switching oligonucleotide under conditions which permit template dependent extension of said cDNA, such that the 3' end of the cDNA comprises a sequence complementary to said template switching oligonucleotide;

(e) providing an amplification primer under conditions to generate double stranded amplification products corresponding substantially to the first strand cDNA synthesis, such that the cDNA amplification products comprise a double stranded RNA polymerase promoter; and

(f) incubating said cDNA amplification products comprising said double stranded RNA polymerase promoter under conditions that permit *in vitro* transcription to generate amplified RNA.

19. A method according to claim 18 wherein said cDNA-RNA hybrid is incubated with a reverse transcriptase that adds at least one deoxycytidine residue to the 3' end of the first strand cDNA synthesis.

20. A method according to claim 18 or claim 19 wherein at least three non-templated nucleotide at the 3' end of the first strand cDNA synthesis are hybridised to a template switching oligonucleotide.

21. A method according to any one of claims 18 to 20 wherein the at least three of the non-templated nucleotides at the 3' end of the first strand cDNA synthesis are deoxycytidine residues.

22. A method according to any one of claims 18 to 21 wherein the reverse transcriptase lacks RNaseH activity but retains wild-type polymerase activity.

23. A method according to any one of claims 18 to 22 wherein the reverse transcriptase is a Moloney Murine Leukemia virus (MMLV) reverse transcriptase or a mutant thereof.

24. A method according to any one of claims 18 to 23 wherein the reverse transcriptase is PowerScript™ Reverse Transcriptase (BD Biosciences Clontech).

25. A method according to any one of claims 18 to 24 wherein said template switching oligonucleotide comprises at least three ribonucleotide residues.

26. A method according to any one of claims 18 to 25 wherein said template switching oligonucleotide comprises at least three guanine residues.

27. A method according to any one of claims 18 to 26 wherein said amplification primer has the same sequence as the amplifier sequence of said cDNA synthesis oligonucleotide.

28. A method according to any one of claims 18 to 27 wherein the reaction comprises 1 mM dNTPs.

29. A method according to any one of claims 18 to 28 wherein the double stranded amplification products are obtained by PCR.

30. A method according to any one of claims 18 to 29 wherein the cDNA synthesis oligonucleotide and the PCR primer have the same concentration

31. A method according to any one claims 18 to 30 wherein PCR amplification is performed using Advantage® 2 Polymerase mix (BD Biosciences Clontech).

32. A method according to any one of claims 18 to 31 wherein the optimum number of cycles to generate the double stranded amplification products is determined by a method comprising the steps of:

(a) providing a plurality of samples with a known amount of RNA;

(b) performing amplification for a defined number of cycles on the plurality of samples;

(c) purifying the double stranded amplification products;

(d) providing for the *in vitro* transcription of the purified amplification products; and

(e) determining the number of amplification cycles that results in the minimum amount of amplified RNA that is required.

33. A method according to any one of claims 18 to 32 wherein the RNA sample is a clinical sample selected from the group consisting of a biopsy, a microdissected tissue, a fine needle aspirate, a flow-sorted cell, a laser captured microdissected cell or a single cell.

34. A method for preparing an expression library of a cell or a cell population comprising the steps of:

(a) providing a cDNA synthesis oligonucleotide comprising an amplifier sequence and an RNA annealing region operably linked to an RNA polymerase promoter

(b) contacting said cDNA synthesis oligonucleotide with a population of mRNAs from said cell or cell population under conditions to allow hybridisation of said cDNA synthesis oligonucleotide to mRNA to produce a cDNA-mRNA complex;

(c) incubating said cDNA-mRNA complex under conditions which permit template-dependent extension of said cDNA synthesis oligonucleotide to generate a cDNA-mRNA hybrid;

(d) contacting said cDNA-mRNA hybrid with a template switching oligonucleotide under conditions which permit template dependent extension of said cDNA, such that the 3' end of

the cDNA of the cDNA-mRNA hybrid comprises a sequence complementary to said template switching oligonucleotide;

(e) contacting an amplification primer with said cDNA-mRNA hybrid under conditions that generate double stranded amplification products corresponding to the first strand cDNA synthesis, such that the double stranded cDNA amplification products comprise a double stranded RNA polymerase promoter; and

(f) incubating said double stranded cDNA amplification products comprising said double stranded RNA polymerase promoter under conditions that permit *in vitro* transcription to generate amplified RNA.

35. A method of preparing a cDNA library from a collection of mRNA molecules comprising the steps of:

(a) providing a cDNA synthesis oligonucleotide comprising an amplifier sequence and an RNA annealing region operably linked to an RNA polymerase promoter;

(b) contacting said cDNA synthesis oligonucleotide with the collection of mRNAs under conditions to allow annealing of said cDNA synthesis oligonucleotide to mRNA produce a cDNA-mRNA complex;

(c) incubating said cDNA-mRNA complex under conditions which permit template-dependent extension of said cDNA synthesis oligonucleotide to generate a cDNA-mRNA hybrid;

(d) contacting said cDNA-mRNA hybrid with a template switching oligonucleotide under conditions which permit template dependent extension of said cDNA of said hybrid, such that the 3' end of the cDNA of the cDNA-mRNA hybrid comprises a sequence complementary to said template switching oligonucleotide;

(e) contacting a PCR primer with said cDNA-mRNA hybrid under conditions that generate double stranded amplification products corresponding to the first strand cDNA

synthesis, such that the double stranded cDNA amplification products comprise a double stranded RNA polymerase promoter;

(f) incubating said double stranded cDNA amplification products comprising said double stranded RNA polymerase promoter under conditions that permit *in vitro* transcription to generate amplified RNA; and

(g) preparing a cDNA library from the amplified RNA.

36. A method for performing subtractive hybridisation comprising the steps of:

(a) providing a cDNA synthesis oligonucleotide comprising an amplifier sequence and an RNA annealing region operably linked to an RNA polymerase promoter;

(b) contacting the cDNA synthesis oligonucleotide with a collection of mRNAs under conditions to allow annealing of said cDNA synthesis oligonucleotide to mRNA in said RNA sample to produce a cDNA-mRNA complex;

(c) incubating said cDNA-mRNA hybrid with enzyme, dNTPs and buffer under conditions which permit template-dependent extension of said cDNA synthesis oligonucleotide to generate a cDNA-mRNA hybrid;

(d) contacting said cDNA-mRNA hybrid with a template switching oligonucleotide under conditions which permit template dependent extension of said cDNA of said hybrid, such that the 3' end of the cDNA of the cDNA-mRNA hybrid comprises a sequence complementary to said template switching oligonucleotide;

(e) contacting an amplification primer with said cDNA-mRNA hybrid under conditions to generate double stranded amplification products corresponding to the first stand cDNA synthesis, such that the double stranded cDNA amplification products comprise a double stranded RNA polymerase promoter;

(f) incubating said double stranded cDNA amplification products comprising said double stranded RNA polymerase promoter under conditions that permit *in vitro* transcription to generate amplified RNA;

(g) contacting said amplified RNA with a single stranded nucleic acid population in the opposite sense to said amplified RNA;

(h) providing for the hybridisation of the sequences present in the amplified RNA and the single stranded nucleic acid population; and

(i) isolating the nucleic acid population that remains single stranded.

37. A method for detecting the expression of a gene of interest comprising the steps of:

(a) providing a cDNA synthesis oligonucleotide comprising an amplifier sequence and an RNA annealing region operably linked to an RNA polymerase promoter, wherein the RNA annealing region comprises a sequence that is substantially homologous to the mRNA expressed by the gene of interest;

(b) contacting said cDNA synthesis oligonucleotide with a population of mRNAs in a cell or cell population under conditions to allow annealing of said cDNA synthesis oligonucleotide to mRNA to produce a cDNA-mRNA complex;

(c) incubating said cDNA-mRNA hybrid under conditions which permit template-dependent extension of said cDNA synthesis oligonucleotide to generate a cDNA-mRNA hybrid;

(d) contacting said cDNA-mRNA hybrid with a template switching oligonucleotide under conditions which permit template dependent extension of said cDNA of said hybrid, such that the 3' end of the cDNA of the cDNA-mRNA hybrid comprises a sequence complementary to said template switching oligonucleotide;

(e) contacting an amplification primer with said cDNA-mRNA hybrid under conditions to generate double stranded amplification products corresponding to the first stand cDNA synthesis, such that the double stranded cDNA amplification products comprise a double stranded RNA polymerase promoter;

(f) incubating said double stranded cDNA amplification products comprising said double stranded RNA polymerase promoter under conditions that permit *in vitro* transcription to generate amplified RNA; and

(g) determining the presence or absence of amplified RNA, which amplified RNA is complementary to mRNA corresponding to the gene of interest.

38. Amplified RNA obtainable by the method according to any one of claims 18 to 33.

39. An expression library obtainable by the method according to claim 34.

40. A cDNA library obtainable by the method according to claim 35.

41. Use of a cDNA-mRNA hybrid according to any one of claims 1-17 in the amplification of RNA.

42. Use of a cDNA-mRNA hybrid according to any one of claims 1-17 in the preparation of a cDNA library.

43. Use of a cDNA-mRNA hybrid according to any one of claims 1-17 in subtractive hybridisation.

44. Use of a cDNA-mRNA hybrid according to any one of claims 1-17 for measuring gene expression.

45. The use according to claim 44 wherein the gene expression is measured using a microarray.

46. A kit for the amplification of RNA in a sample comprising:

(a) a cDNA synthesis oligonucleotide comprising an amplifier sequence and an RNA annealing region operably linked to an RNA polymerase promoter;

(b) a template switching oligonucleotide that has substantially the same sequence as the amplifier sequence; and

(c) an amplification primer that has substantially the same sequence as the template switching oligonucleotide.

47. The kit according to claim 46, wherein the kit further comprises in a separate container a reverse transcriptase.

48. The kit according to claim 47 wherein the reverse transcriptase lacks RNaseH activity but retains wild-type polymerase activity.

49. The kit according to claim 47 or claim 48 wherein the reverse transcriptase is a Moloney Murine Leukemia virus (MMLV) reverse transcriptase or a mutant thereof.

50. The kit according to any one of claims claim 47 to 49 wherein the reverse transcriptase is PowerScript™ Reverse Transcriptase (BD Biosciences Clontech).

51. The kit according to any one of claims 47 to 50, wherein the kit further comprises in a separate container an RNA polymerase specific to the RNA polymerase promoter of the cDNA synthesis oligonucleotide.

52. The kit according to any one of claims claim 47 to 51, wherein the RNA polymerase promoter is selected from a T7, T3 or SP6 RNA polymerase promoter.

53. The kit according to any one of claims 47 to 52, wherein the kit further comprises an amplification buffer and one or more amplification enzymes.

54. The kit according to claim 53, wherein the amplification buffer and the amplification enzyme(s) are PCR amplification buffer and PCR amplification enzyme(s).

55. The kit according to any one of claims 47 to 54, wherein the kit further comprises a control nucleic acid.